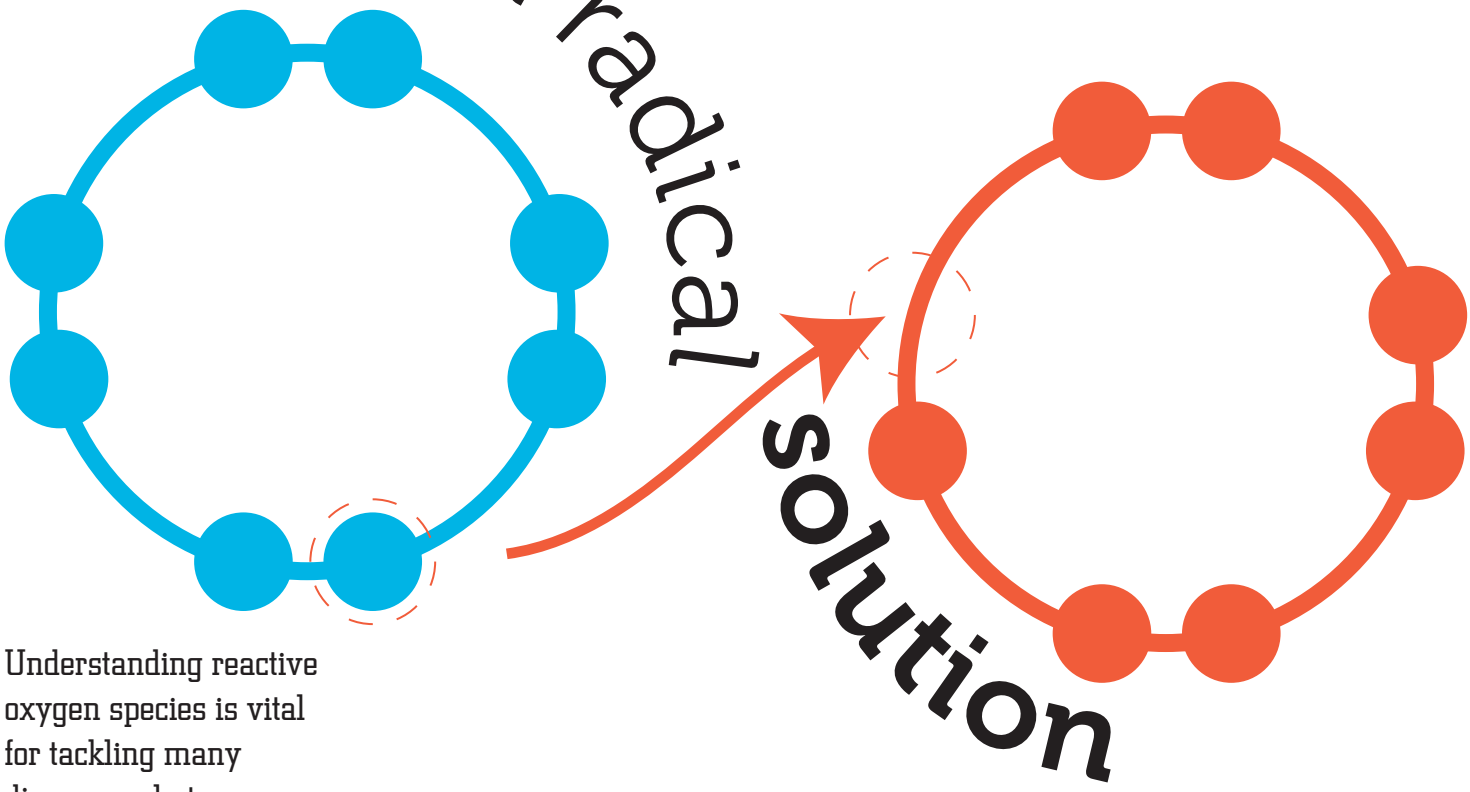
A radical solution



Understanding reactive oxygen species is vital for tackling many diseases – but you need to catch them first. Here **Dr Kalina Ranguelova** talks us through this challenge and a possible solution in the shape of electron paramagnetic resonance

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The development of diseases such as cancer, cardiovascular diseases, atherosclerosis, autism, infections, Alzheimer's and Parkinson's disease are commonly attributed to oxidative stress and cell damage. These disease factors are mainly caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS) which are highly reactive molecules and free radicals derived from molecular oxygen or nitrogen that can cause damage in cells, proteins, lipids, RNA and DNA.

Electron paramagnetic resonance (EPR) is most commonly used for the detection and characterisation of free radicals in biological samples, in order to determine their role in physiological or pathophysiological processes. Most ROS and RNS are short-lived and are therefore difficult to detect in biological samples. Direct detection of ROS and RNS is very difficult or impossible in solution at room temperature due to their very short half-life. For this reason, EPR spin trapping technique is used.

Free radicals are intermediates in a wide range of biochemical reactions, and have drawn increasing interest over recent years. Example of ROS compounds are hydroxyl, hydroperoxyl and superoxide radicals, all of which are produced in natural biological reactions. RNS such as nitrogen monoxide and peroxynitrite are also commonly produced. ROS and RNS levels can become excessive and unbalanced and regulating their levels is beneficial in designing novel therapies for disease.[1]

Regardless of the target application, every EPR spectrometer needs to be highly sensitive and stable. Advances in magnet and microwave technology contribute to enhanced performance, addressing the needs of researchers for both ease of use with high quality EPR data. The interest in use of EPR for analytical chemistry, materials science and biology, has been boosted with the advent of new applications. The technique can be used in static and dynamic investigations of these systems (materials, chemicals and biological systems) the latter of which is beneficial for obtaining an EPR spectrum whole applying changing conditions, such as temperature or light irradiation.

The following examples demonstrate how EPR spectroscopy can be utilised for the detection and characterisation of radicals in biological systems.

Spin trapping

Due to the short half-life of ROS and RNS, direct detection is impossible in solution at room temperature. The generation and decomposition of two leading ROS, the superoxide radical (O_2•–) and the hydroxyl radical (•OH) can be accurately followed using quantitative EPR.

EPR spin trapping was developed in the late 1960s, where a nitrone or nitroso compound reacts with a target free radical to form a stable and distinguishable free radical that is detected by EPR spectroscopy. By adding the reactive free radical to the double bond of a diamagnetic 'spin trap', a much more stable 'radical adduct' is formed, which has individual features enabling the originally generated reactive radical to be easily identified.

Spin probes

It has been suggested that, in vascular cells, increased generation of superoxide (O_2•–) occurs in hypertension, diabetes, and heart failure. Thus the ability to accurately detect and quantify O_2•– is crucial to the understanding of pathogenesis of these diseases. The generation of superoxide over time can be easily monitored with a bench-top EPR spectrometer, despite the usual difficulties in direct detection of ROS and RNS.

Spin probes are not spin traps, in that they do not 'trap' radicals, rather they are oxidised by ROS or RNS to form nitroxides (free radicals) with a half-life of several hours, which can readily be detected by EPR. For example, the cyclic hydroxylamine 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH) can provide quantitative measurements of superoxide (O_2•–) with high sensitivity and it has been used for detection of intracellular O_2•– in cultured cells and tissue samples.

Bench-top EPR spectrometers can be used effectively for mechanistic studies and kinetic analysis of multiple radicals (ROS and RNS) generated in enzyme reactions. Adequately controlled spin probe experiments can verify that the formation of radical adducts is due to free radical production in the reaction system being studied.

NO trapping

Human skin contains photolabile nitric oxide (NO) derivatives which, upon UVA radiation, decompose under high-output NO formation and exert NO-specific biological responses such as increased local blood flow or reduced blood pressure. The intradermal increase of free NO due to blue light irradiation of human skin can be monitored and quantified using EPR.

NO is a highly reactive regulatory molecule with many important physiological roles, such as a neurotransmitter in the central nervous system, a regulator of vasomotor tone in the cardiovascular system, and a cytotoxic mediator of the immune system. NO is an unstable free radical, with a short half-life (<30 sec) which causes difficulties in direct measurement. This can be mitigated by using a NO-trapping technique, where a more stable complex is formed and subsequently detected by EPR. For example, the oxidation of nitric oxide (NO) to nitrate by oxyhemoglobin (oxyHb) is a fundamental reaction in NO biology and binding of NO to the heme can be characterised by EPR.

With accessories for high-sensitivity room temperature and low temperature measurements, detection of NO with MGD or hemoglobin as a spin trap is easy to achieve with EPR.

Bench-top EPR

User-centric bench-top EPR spectrometers have been designed for those who require research performance and ease of use. Such an instrument provides many features typically found only on sophisticated, floor-standing EPR instruments, making research-grade EPR capabilities accessible to a broader range of scientists. Bench-top instruments often include defined workflows for easy and fast system setup, with user friendly interfaces that allow parameters to be easily adjusted also by non-EPR experts.

They can be used to analyse many EPR samples, including transition metals, antioxidants and free radicals, providing valuable information and insights into biological and chemical systems.

Discovering novel cures for illnesses is becoming increasingly pressing, given the high mortality rates and increased costs to the healthcare system associated with widespread diseases. Given the role that free radicals play in the dynamics of biological systems, EPR is rapidly evolving as the 'gold standard' spectroscopy technique. High-quality information on the structure, mobility and interactions of free radical species enables researchers in areas from oncology to autism and Parkinson's disease to better understand the mechanisms of disease.

EPR remains a definitive method of identifying radicals in complex systems and is also a valuable method of examining radical kinetics, concentrations and structure. In the near future, these innovations will empower researchers with robust data, further accelerating the development of drugs and informing clinical practice.

From membranes to solar cells – EPR applications spread far and wide...

EPR is the only method available for the direct detection of paramagnetic ROS and RNS species. It has led to the understanding of metalloprotein structures and the processes involved in photosynthesis.

In biology, EPR can be applied to the study of membrane proteins, metalloenzymes, IDPs, RNA, DNA, spin labelling/trapping, nitric oxides and ROS & RNS. Further applications include polymer synthesis, testing the purity of silicon in solar cells, spin trapping to assess the oxidative stability of flavours, and the analysis of metalloproteins.

In electrochemistry, redox chemistry, photochemistry and catalysis, EPR can be used to study metal centres and radicals involved in chemical processes.

1. *Rouaud, F. et al., (2014) Regulation of NADPH-dependent Nitric Oxide and reactive oxygen species signalling in endothelial and melanoma cells by a photoactive NADPH analogue, Oncotarget 5 (21): 10650 – 10664.*